

oligonucleotide sequences being chosen to sample a length of said nucleotide sequence,

(c) memory means for storing said oligonucleotide sequences,

(d) means for controlling said computer system to carry out a determination and evaluation for each of said oligonucleotide sequences a value for at least one parameter that is predictive of the ability of each of said oligonucleotide sequences to hybridize to said target nucleotide sequence,

(e) means for storing said parameter values,

(f) means for controlling said computer system to carry out an identification, from said stored parameter values, a subset of oligonucleotide sequences within said number of unique oligonucleotide sequences based on an examination of said parameter,

(g) means for storing said subset of oligonucleotide sequences,

(h) means for controlling said computer system to carry out an identification of oligonucleotide sequences in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence,

(i) means for storing said oligonucleotide sequences in said subset,

(j) means for controlling said computer system to select, for a cluster, a hybridization oligonucleotide and

(k) means for outputting data relating to said oligonucleotide sequences in said subset.

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cont

REMARKS

Applicants request reconsideration of their application in view of the foregoing amendments and the discussion that follows. The specification has been amended herein to refer to the Sequence Listing on compact disk and to insert sequence identification numbers for all the sequences set forth. The claims have also been amended as indicated above.

Attached hereto is a marked-up version of the changes made to the specification and the claims by the current amendment. The attached pages are captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE." Also attached are substitute pages 46-50 for use by the Examiner as well as a clean copy of claims 1-40 and 98-101.

The Amendment

The specification was amended to refer to the computer program listing appendix on compact disk. Furthermore, as mentioned above, the specification has been amended to insert sequence identification numbers for all the sequences set forth. As can readily be seen, the target complement sequence, which appears on page 45 and is designated SEQ ID NO: 9, is repeated on pages 46-50 and the sequences designated SEQ ID NO: 10 to SEQ ID NO: 35 on page 46 are repeated on pages 47-50.

Claim 1 was amended to delete the word "entire" in step (a) and the word "independently" in step (b). Support therefor is in the Specification, for example, page 29, line 31, to page 30, line 2. Step (c) was amended to recite "selecting" instead of "identifying." Support therefor is in the Specification, for example, original Claim 1. Step (d) was amended to recite "in clusters" instead of "clustered." Support therefor is in the Specification, for example, original Claim 1. Step (e) was added and recites that a hybridization oligonucleotide is selected for a cluster. Support therefor is in the Specification, for example, page 30, lines 6-8.

Claims 98 and 100 were amended in a manner similar to that for Claim 1.

Objection by Draftsperson

Enclosed herewith is a corrected set of drawings wherein Figs. 2-8 have corrected left margins and Fig. 2 has shading removed. No other changes have been made to the drawings as originally submitted.

Sequence Compliance

As mentioned above, the specification has been amended to insert sequence identification numbers for all the sequences set forth. The target complement sequence, which appears on page 45 and is designated SEQ ID NO: 9, is repeated on pages 46-50 and the sequences designated SEQ ID NO: 10 to SEQ ID NO: 35 on page 46 are repeated on pages 47-50. All of these sequences are already included in the paper copy of the sequence submitted with the filed application as well as in the computer readable form of the sequence listing. With respect to the latter, Applicant previously submitted a request in the form of a letter in accordance with 37 C.F.R. 1.821(e) making reference to the parent application for the above-referenced application as well as the sequence listing in computer readable form and a request

to use the compliant computer readable "Sequence Listing" that is on file for the parent application.

Restriction/Election Requirement

The Examiner required restriction to one of the following inventions:

Group I – Claims 1-40 and 98-101

Group II – Claims 41-97

In response thereto Applicant elects the invention of Group I, namely, Claims 1-40 and 98-101.

The Examiner indicated that the various inventions are patentably distinct. Accordingly, the Examiner has determined that the inventions of the various groups are separately patentable over one other. According to M.P.E.P. 802.01 the term "distinct" means that two or more subjects as disclosed are related, for example, as combination and part (subcombination) thereof, process and apparatus for its practice, process and product made, etc., but are capable of separate manufacture, use, or sale as claimed, AND ARE PATENTABLE (novel and unobvious) OVER EACH OTHER (emphasis in original). Accordingly, the restriction/election requirement necessarily involved the Examiner's determination at least implicitly that the inventions of the various groups are separately patentable over one other. If this were not the case, then the restriction/election requirement would not be appropriate.

The Examiner indicated further that, if Group I were elected, then the species elections set forth in the Office Action would be required. The species elections were phrased as being "applicable only if Group I or Group II is elected." Applicant is unable to find any groups other than Groups I and II. Accordingly, Applicant is confused by the above language and respectfully asks for clarification.

In response to the requirement for election as it is presently understood, Applicant elects the species of Group IB, namely, thermodynamic factors as in Claims 5, 7, 47, 49, 78, 79 and 82. Furthermore, Applicant elects the species of Group IIA, namely, non-chemically modified nucleotides as in Claims 17, 18, 20, 21, 60, 61, 63, 64, 87, 88, 90 and 91. Claims 1-16, 23-59, 66-86 and 93-101 are also generic to the aforementioned species. The above respective claims that are in elected Group I constitute the claims readable on the aforementioned species.

The Examiner further required that Applicant elect a single disclosed species, for each of the above species set forth above, for prosecution on the merits. For the

species of Group IB, namely, thermodynamic factors, Applicant elects the species set forth at page 45, line 8, namely, duplex melting temperature. The claims of Group I that are readable on this species are Claims 5 and 7, as well as those claims indicated by the Examiner as being generic to all of the species. For the species of Group IIA, namely, non-chemically modified nucleotides, Applicant elects the species DNA as set forth in Claim 17. The claims readable on this species are Claims 17, 21 and 23, as well as those claims indicated by the Examiner as being generic to all of the species.

It has been held that a requirement for election of species is tantamount to a restriction requirement. Accordingly, Applicant reserves the right to file divisional patent applications to all of the species that the Examiner has determined are patentable over one another. See also M.P.E.P. 806.04(h).

CONCLUSION

The specification has been amended to include sequence identification numbers for all sequences in the specification. All such sequences were repetitions of sequences that were given sequence identification numbers in the originally filed application. Corrected drawings, which correct the informalities noted by the Draftsperson, are submitted herewith. Appropriate election of invention and election of species have been made.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADEIn the Specification

The specification has been amended as follows:

The paragraph on page 1, lines 23-25, after the title "Appendix" was replaced with the following amended paragraph:

This patent application includes [an] a computer program listing appendix (Appendix), which contains the source code for the software used in carrying out the examples in accordance with the present invention. The Appendix is contained on one compact disc submitted in duplicate and designated as Copy 1 and Copy 2. The Appendix is in a single file that is 292 kB in size and designated "computer program listing appendix U.S. Serial No. 09-021,721". The file was created on 02/02/1998 and is a Microsoft Word document. The material in the Appendix is incorporated herein by reference.

The paragraphs beginning on page 46, line 4, to page 50, line 35, have been replaced with the following amended paragraphs:

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO: 11
CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
CAAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO: 14
AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO: 18
GGTCAGTCTACCTCCCGCCATAAAA	76.85	-1.20	SEQ ID NO: 19
GGTCAGTCTACCTCCCGCCATAAAAA	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTT	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
CCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Next, the oligonucleotide sequences are filtered on the basis of T_m . A high and low cut-off value may be selected, for example, $60^\circ\text{C} \leq T_m \leq 85^\circ\text{C}$. Thus, oligonucleotides having T_m values falling within the above range are retained. Those outside the range are discarded, which is indicated below by lining out of those oligonucleotides and parameter values.

GTCCAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m ($^\circ\text{C}$)	ΔG_{MFOLD}	
GTCCAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO: 11
CCAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO: 14
AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO: 18
GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20	SEQ ID NO: 19
GGTCAGTCTACCTCCCGCCATAAAA	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
CCGECATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Next, the oligonucleotide sequences remaining after the above exercise are filtered on the basis of ΔG_{MFOLD} and are retained if the value is greater than -0.4. Those oligonucleotides with a ΔG_{MFOLD} less than -0.4 are discarded, which is indicated below by double lining out of those oligonucleotides and parameter values.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAAGGGTCAGTCTACCTCCG	71.99	-1.20	SEQ ID NO: 11
CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
CAAAAAGGGTCAGTCTACCTCCCGG	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCCCGGG	73.07	-1.20	SEQ ID NO: 14
AAAAAGGGTCAGTCTACCTCCCGGCA	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCCCGGCAAT	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCCCGGCGATA	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCCCGGCGATAA	79.03	-1.20	SEQ ID NO: 18
GGGTCAGTCTACCTCCCGGCGATAAA	76.85	-1.20	SEQ ID NO: 19
GGTGAGTCTACCTCCCGGCGATAAAA	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTC	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
CCGCGATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
CGCGATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Clusters of retained oligonucleotides are identified and ranked based on cluster size. In this example a contiguous cluster of 13 retained oligonucleotides is identified by the vertical black bar on the left. All of the oligonucleotides in this cluster may be evaluated experimentally.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
TCCAAAAAGGGTCAGTCTACCTCC	71.99	-1.20	SEQ ID NO: 11
CCAAAAAGGGTCAGTCTACCTCC	70.78	-1.20	SEQ ID NO: 12
CAAAAAAGGGTCAGTCTACCTCC	71.23	-1.20	SEQ ID NO: 13
AAAAAGGGTCAGTCTACCTCC	73.07	-1.20	SEQ ID NO: 14
AAAAAGGGTCAGTCTACCTCC	75.68	-1.20	SEQ ID NO: 15
AAAGGGTCAGTCTACCTCC	77.53	-1.20	SEQ ID NO: 16
AAGGGTCAGTCTACCTCC	79.03	-1.20	SEQ ID NO: 17
AGGGTCAGTCTACCTCC	79.03	-1.20	SEQ ID NO: 18
GGGTCAGTCTACCTCC	76.85	-1.20	SEQ ID NO: 19
GTCAGTCTACCTCCCGCCATAAAAA	73.10	-0.80	SEQ ID NO: 20
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
TCAGTCTACCTCCCGCCATAAAAA	65.60	0.90	SEQ ID NO: 22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10	SEQ ID NO: 31
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
CCCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
CCCGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Alternatively, in one approach the oligonucleotides at the first quartile, the median and the third quartile of the cluster may be selected for experimental evaluation, indicated below by bold print.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	71.77	-1.20	<u>SEQ ID NO: 10</u>
TCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	71.99	-1.20	<u>SEQ ID NO: 11</u>
CGAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	70.78	-1.20	<u>SEQ ID NO: 12</u>
GAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	71.23	-1.20	<u>SEQ ID NO: 13</u>
AAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	73.07	-1.20	<u>SEQ ID NO: 14</u>
AAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	75.68	-1.20	<u>SEQ ID NO: 15</u>
AAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	77.53	-1.20	<u>SEQ ID NO: 16</u>
AAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	79.03	-1.20	<u>SEQ ID NO: 17</u>
AGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	79.03	-1.20	<u>SEQ ID NO: 18</u>
GGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	76.85	-1.20	<u>SEQ ID NO: 19</u>
GTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	73.10	-0.00	<u>SEQ ID NO: 20</u>
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	<u>SEQ ID NO: 21</u>
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	<u>SEQ ID NO: 22</u>
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	<u>SEQ ID NO: 23</u>
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	<u>SEQ ID NO: 24</u>
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	<u>SEQ ID NO: 25</u>
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	<u>SEQ ID NO: 26</u>
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	<u>SEQ ID NO: 27</u>
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	<u>SEQ ID NO: 28</u>
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	<u>SEQ ID NO: 29</u>
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	<u>SEQ ID NO: 30</u>
CTCCCGCCATAAAAAACTCATGTTT	63.37	-0.10	<u>SEQ ID NO: 31</u>
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	<u>SEQ ID NO: 32</u>
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	<u>SEQ ID NO: 33</u>
CGCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	<u>SEQ ID NO: 34</u>
CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	<u>SEQ ID NO: 35</u>

In the Claims

The claims have been amended as follows:

1. **(amended)** A method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a [the entire] length of said nucleotide sequence,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is [independently] predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence,

(c) selecting ~~[identifying]~~ a subset of oligonucleotides within said predetermined number of unique oligonucleotides based on an examination of said parameter, ~~[and]~~

(d) identifying oligonucleotides in said subset that are in clusters ~~[clustered]~~ along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) selecting, for a cluster, a hybridization oligonucleotide.

98. **(amended)** A computer based method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying under computer control a predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a ~~[the entire]~~ length of said nucleotide sequence,

(b) under computer control, determining and evaluating for each of said oligonucleotides a value for at least one parameter that is ~~[independently]~~ predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence and storing said parameter values,

(c) selecting ~~[identifying]~~ under computer control, from said stored parameter values, a subset of oligonucleotides within said predetermined number of unique oligonucleotides based on an examination of said parameter, ~~[and]~~

(d) identifying under computer control oligonucleotides in said subset that are in clusters ~~[clustered]~~ along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) under computer control selecting, for a cluster, a hybridization oligonucleotide.

100. **(amended)** A computer system for conducting a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) input means for introducing a target nucleotide sequence into said computer system,

(b) means for determining a number of unique oligonucleotides that are within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotide sequences being chosen to sample a [the entire] length of said nucleotide sequence,

(c) memory means for storing said oligonucleotide sequences,

(d) means for controlling said computer system to carry out a determination and evaluation for each of said oligonucleotide sequences a value for at least one parameter that is [independently] predictive of the ability of each of said oligonucleotide sequences to hybridize to said target nucleotide sequence,

(e) means for storing said parameter values,

(f) means for controlling said computer system to carry out an identification, from said stored parameter values, a subset of oligonucleotide sequences within said number of unique oligonucleotide sequences based on an examination of said parameter,

(g) means for storing said subset of oligonucleotide[s] sequences,

(h) means for controlling said computer system to carry out an identification of oligonucleotide sequences in said subset that are in clusters [clustered] along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence,

(i) means for storing said oligonucleotide sequences in said subset, [and]

(j) means for controlling said computer system to select, for a cluster, a hybridization oligonucleotide and

(k) means for outputting data relating to said oligonucleotide sequences in said subset.

CLEAN COPY OF CLAIMS 1-40 AND 98-101

1. **(amended)** A method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a length of said nucleotide sequence,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence,

(c) selecting a subset of oligonucleotides within said predetermined number of unique oligonucleotides based on an examination of said parameter,

(d) identifying oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) selecting, for a cluster, a hybridization oligonucleotide.

2. A method according to Claim 1 which comprises ranking said oligonucleotides of step (d) based on the size of said clusters of oligonucleotides.

3. A method according to Claim 1 wherein said unique oligonucleotides are of identical length N.

4. A method according to Claim 3 wherein said unique oligonucleotides are spaced one nucleotide apart, said predetermined number comprising $L-N+1$ oligonucleotides, where L is the length of the hybridizable sequence.

5. A method according to Claim 1 wherein said parameter is selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies and kinetic factors.

6. A method according to Claim 1 wherein said parameter is a composition factor selected from the group consisting of mole fraction (G+C), percent (G+C), sequence complexity, and sequence information content.

7. A method according to Claim 1 wherein said parameter is a thermodynamic factor selected from the group consisting of predicted duplex melting temperature, predicted enthalpy of duplex formation, predicted entropy of duplex formation, predicted free energy of duplex formation, predicted melting temperature of the most stable intramolecular structure of the oligonucleotide or its complement, predicted enthalpy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted entropy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted free energy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted melting temperature of the most stable hairpin structure of the oligonucleotide or its complement, predicted enthalpy of the most stable hairpin structure of the oligonucleotide or its complement, predicted entropy of the most stable hairpin structure of the oligonucleotide or its complement, predicted free energy of the most stable hairpin structure of the oligonucleotide or its complement, thermodynamic partition function for intramolecular structure of the oligonucleotide or its complement.

8. A method according to Claim 1 wherein said parameter is a chemosynthetic efficiency selected from the group consisting of coupling efficiencies and overall efficiency of the synthesis of a target nucleotide sequence or an oligonucleotide probe.

9. A method according to Claim 1 wherein said parameter is a kinetic factor selected from the group consisting of steric factors calculated via molecular modeling, rate constants calculated via molecular dynamics simulations, rate constants calculated via semi-empirical kinetic modeling, associative rate constants, dissociative rate constants, enthalpies of activation, entropies of activation, and free energies of activation.

10. A method according to Claim 1 wherein said parameter is derived from a factor by mathematical transformation of said factor.

11. A method according to Claim 1 which comprises ranking said clustered oligonucleotides of step (d) based on the size of said clusters of oligonucleotides and selecting a subset of said clustered oligonucleotides.

12. A method according to Claim 11 wherein said subset consists of any number of oligonucleotides within said cluster of oligonucleotides.

13. A method according to Claim 11 wherein the subset of said clustered oligonucleotides are selected to statistically sample the cluster.
14. A method according to Claim 13 wherein said statistical sample consists of oligonucleotides spaced at the first quartile, median and third quartile of the cluster of oligonucleotides.
15. A method according to Claim 1 wherein said parameters are determined for said oligonucleotides by means of a computer program.
16. A method according to Claim 1 wherein said oligonucleotides are attached to a surface.
17. A method according to Claim 1 wherein said oligonucleotides are DNA.
18. A method according to Claim 1 wherein said oligonucleotides are RNA.
19. A method according to Claim 1 wherein said oligonucleotides contain chemically modified nucleotides.
20. A method according to Claim 1 wherein said target nucleotide sequence is RNA.
21. A method according to Claim 1 wherein said target nucleotide sequence is DNA.
22. A method according to Claim 1 wherein said target nucleotide sequence contains chemically modified nucleotides.
23. A method according to Claim 1 wherein said parameter is, for each oligonucleotide/target nucleotide sequence duplex, the difference between the predicted duplex melting temperature corrected for salt concentration and the temperature of hybridization of each of said oligonucleotides with said target nucleotide sequence.

24. A method according to Claim 1 wherein step (c) comprises identifying a subset of oligonucleotides within said predetermined number of unique oligonucleotides by establishing cut-off values for said parameter.

25. A method according to Claim 1 wherein said step (c) comprises identifying a subset of oligonucleotides within said predetermined number of unique oligonucleotides by converting the values of said parameter into a dimensionless number.

26. A method according to Claim 25 wherein said value is converted into a dimensionless number by determining a dimensionless score for each parameter resulting in a distribution of scores having a mean value of zero and a standard deviation of one.

27. A method according to Claim 26 which comprises optimizing a method according to calculation for said parameter based on said individual scores.

28. A method according to Claim 1 wherein step (b) comprises determining at least two parameters wherein said parameters are poorly correlated with respect to one another.

29. A method according to Claim 28 wherein said parameters are derived from a combination of factors by mathematical transformation of those factors.

30. A method according to Claim 1 wherein step (b) comprises determining two parameters at least one of said parameters being the association free energy between a subsequence within each of said oligonucleotides and its complementary sequence on said target nucleotide sequence.

31. A method according to Claim 30 wherein said subsequence is 3 to 9 nucleotides in length.

32. A method according to Claim 30 wherein said subsequence is 5 to 7 nucleotides in length.

33. A method according to Claim 30 wherein said subsequence is at least three nucleotides from the terminus of said oligonucleotides.

34. A method according to Claim 30 wherein said subsequence is at least three nucleotides from a surface to which said oligonucleotides are attached.

35. A method according to Claim 30 wherein said oligonucleotides are attached to a surface and said subsequence is at least five nucleotides from the terminus of said oligonucleotides that is attached to said surface and at least three nucleotides from the free end of said oligonucleotides.

36. A method according to Claim 30 wherein the association free energy of the members of a set of subsequences within each of said oligonucleotides is determined and said subsequence having the minimum value is identified.

37. A method according to Claim 1 which comprises including oligonucleotides that are adjacent to said oligonucleotides in said subset that are clustered along a region of said target nucleotide sequence.

38. A method according to Claim 1 which comprises (i) identifying a subset of oligonucleotides within said predetermined number of unique oligonucleotides by establishing cut-off values for each of said parameters.

39. A method according to Claim 1 which comprises determining the sizes of said clusters of step (d) by counting the number of contiguous oligonucleotides in said region of said hybridizable sequence.

40. A method according to Claim 1 which comprises determining the sizes of said clusters of step (d) by counting the number of oligonucleotides in said subset that begin in a region of predetermined length in said hybridizable sequence.

98. **(amended)** A computer based method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying under computer control a predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a length of said nucleotide sequence,

(b) under computer control, determining and evaluating for each of said oligonucleotides a value for at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence and storing said parameter values,

(c) selecting under computer control, from said stored parameter values, a subset of oligonucleotides within said predetermined number of unique oligonucleotides based on an examination of said parameter,

(d) identifying under computer control oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) under computer control selecting, for a cluster, a hybridization oligonucleotide.

99. A method according to claim 98 wherein the identified subset of oligonucleotide sequences is electronically transferred to an oligonucleotide array manufacturing system.

100. **(amended)** A computer system for conducting a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) input means for introducing a target nucleotide sequence into said computer system,

(b) means for determining a number of unique oligonucleotides that are within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotide sequences being chosen to sample a length of said nucleotide sequence,

(c) memory means for storing said oligonucleotide sequences,

(d) means for controlling said computer system to carry out a determination and evaluation for each of said oligonucleotide sequences a value for at least one parameter that is predictive of the ability of each of said oligonucleotide sequences to hybridize to said target nucleotide sequence,

(e) means for storing said parameter values,

(f) means for controlling said computer system to carry out an identification, from said stored parameter values, a subset of oligonucleotide sequences within said number of unique oligonucleotide sequences based on an examination of said parameter,

(g) means for storing said subset of oligonucleotide sequences,

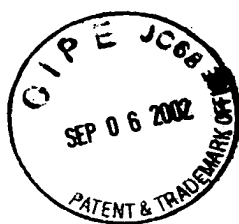
(h) means for controlling said computer system to carry out an identification of oligonucleotide sequences in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence,

(i) means for storing said oligonucleotide sequences in said subset,

(j) means for controlling said computer system to select, for a cluster, a hybridization oligonucleotide and

(k) means for outputting data relating to said oligonucleotide sequences in said subset.

101. A computer system according to claim 100 wherein the identified subset of oligonucleotide sequences is electronically transferred to an oligonucleotide array manufacturing system.



structure of a single stranded DNA molecule (see J.A. Jaeger, *et al.*, (1989), *supra*, respectively. The steps are illustrated below.

5 GTCCAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

		T_m (°C)	ΔG_{MFOLD}	
	GTCCAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
	TCCAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO: 11
10	CCAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
	CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO: 13
	AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO: 14
	AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO: 15
	AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO: 16
15	AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO: 17
	AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO: 18
	GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20	SEQ ID NO: 19
	GGTCAGTCTACCTCCCGCCATAAAA	73.10	-0.80	SEQ ID NO: 20
	GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
20	TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
	CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
	GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
	TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
25	CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
	TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
	CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
	CTCCCGCCATAAAAAACTCATGTTT	63.37	-0.10	SEQ ID NO: 31
30	TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
	CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
	CCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
	CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

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Next, the oligonucleotide sequences are filtered on the basis of T_m . A high and low cut-off value may be selected, for example, $60^\circ\text{C} \leq T_m \leq 85^\circ\text{C}$. Thus, oligonucleotides having T_m values falling within the above range are retained. Those outside the range are discarded, which is indicated below by lining out of those oligonucleotides and parameter values.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

		T_m ($^\circ\text{C}$)	ΔG_{MFOLD}	
10	GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
	TCCAAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO: 11
	CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO: 12
	CAAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO: 13
15	AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO: 14
	AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO: 15
	AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO: 16
	AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO: 17
	AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO: 18
20	GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20	SEQ ID NO: 19
	GGTCAGTCTACCTCCCGCCATAAAA	73.10	-0.80	SEQ ID NO: 20
	GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
	TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
	CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
25	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
	GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
	TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
	CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
	TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
30	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
	CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
	CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10	SEQ ID NO: 31
	TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
	CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
35	EGGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
	EGGCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Next, the oligonucleotide sequences remaining after the above exercise are filtered on the basis of ΔG_{MFOLD} and are retained if the value is greater than - 0.4. Those oligonucleotides with a ΔG_{MFOLD} less than - 0.4 are discarded, which is indicated below by double lining out of those oligonucleotides and parameter values.

5	GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)			
10	GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO: 10
	TCCAAAAAGGGTCAGTCTACCTCCG	71.99	-1.20	SEQ ID NO: 11
	CCAAAAAGGGTCAGTCTACCTCCGG	70.78	-1.20	SEQ ID NO: 12
	CAAAAAGGGTCAGTCTACCTCCGGC	71.23	-1.20	SEQ ID NO: 13
	AAAAAGGGTCAGTCTACCTCCGGCC	73.07	-1.20	SEQ ID NO: 14
15	AAAAAGGGTCAGTCTACCTCCGGCCA	75.68	-1.20	SEQ ID NO: 15
	AAAGGGTCAGTCTACCTCCGGCCAT	77.53	-1.20	SEQ ID NO: 16
	AAGGGTCAGTCTACCTCCGGCCATA	79.03	-1.20	SEQ ID NO: 17
	AGGGTCAGTCTACCTCCGGCCATAA	79.03	-1.20	SEQ ID NO: 18
20	GGGTCACTCTACCTCCGGCCATAAA	76.85	-1.20	SEQ ID NO: 19
	GGTCACTCTACCTCCGGCCATAAAA	73.10	-0.80	SEQ ID NO: 20
	GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
	TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
	CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
25	GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
	TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
	CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
	TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
30	CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
	CTCCCGCCATAAAAAACTCATGTTT	63.37	-0.10	SEQ ID NO: 31
	TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
	CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
	CGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
35	CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Clusters of retained oligonucleotides are identified and ranked based on cluster size. In this example a contiguous cluster of 13 retained oligonucleotides is identified by the vertical black bar on the left. All of the oligonucleotides in this cluster may be evaluated experimentally.

GTCCAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

		T_m (°C)	ΔG_{MFOLD}	
10	GTCCAAAAGGGTCAGTCTACCTCG	71.77	-1.20	SEQ ID NO: 10
	TCCAAAAGGGTCAGTCTACCTCGG	71.99	-1.20	SEQ ID NO: 11
	CCAAAAGGGTCAGTCTACCTCGGG	70.78	-1.20	SEQ ID NO: 12
	CAAAAAGGGTCAGTCTACCTCGGGG	71.23	-1.20	SEQ ID NO: 13
15	AAAAAGGGTCAGTCTACCTCGGGGG	73.07	-1.20	SEQ ID NO: 14
	AAAAGGGTCAGTCTACCTCGGGGGA	75.68	-1.20	SEQ ID NO: 15
	AAAGGGTCAGTCTACCTCGGGGGA	77.53	-1.20	SEQ ID NO: 16
	AAGGGTCAGTCTACCTCGGGGGA	79.03	-1.20	SEQ ID NO: 17
	AGGGTCAGTCTACCTCGGGGGA	79.03	-1.20	SEQ ID NO: 18
20	GGGTCAGTCTACCTCGGGGGA	76.85	-1.20	SEQ ID NO: 19
	GTCAGTCTACCTCGGGGGA	73.10	-0.90	SEQ ID NO: 20
	GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO: 21
	TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO: 22
	CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO: 23
25	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10	SEQ ID NO: 24
	GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO: 25
	TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO: 26
	CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO: 27
	TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO: 28
30	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO: 29
	CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO: 30
	CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10	SEQ ID NO: 31
	TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO: 32
	CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO: 33
35	CGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO: 34
	CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35

Alternatively, in one approach the oligonucleotides at the first quartile, the median and the third quartile of the cluster may be selected for experimental evaluation, indicated below by bold print.

5 GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA (target complement sequence) (SEQ ID NO: 9)

		T_m (°C)	ΔG_{MFOLD}	
10	GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	71.77	-1.20	SEQ ID NO: 10
	TCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	71.99	-1.20	SEQ ID NO: 11
	CCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	70.78	-1.20	SEQ ID NO: 12
	CAAAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	71.23	-1.20	SEQ ID NO: 13
	AAAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	73.07	-1.20	SEQ ID NO: 14
	AAAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	75.68	-1.20	SEQ ID NO: 15
15	AAAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	77.53	-1.20	SEQ ID NO: 16
	AAGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	79.03	-1.20	SEQ ID NO: 17
	AGGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	79.03	-1.20	SEQ ID NO: 18
	GGGTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	76.85	-1.20	SEQ ID NO: 19
	GTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	73.10	-0.90	SEQ ID NO: 20
20	GTCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	69.50	0.90	SEQ ID NO: 21
	TCAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	65.60	0.90	SEQ ID NO: 22
	CAGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	64.96	0.90	SEQ ID NO: 23
	AGTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	65.48	1.10	SEQ ID NO: 24
	GTCTACCTCCCGCCATAAAAACTCATGTTCAAGA	66.36	2.40	SEQ ID NO: 25
25	TCTACCTCCCGCCATAAAAACTCATGTTCAAGA	64.97	2.90	SEQ ID NO: 26
	CTACCTCCCGCCATAAAAACTCATGTTCAAGA	63.96	2.70	SEQ ID NO: 27
	TACCTCCCGCCATAAAAACTCATGTTCAAGA	62.58	1.10	SEQ ID NO: 28
	ACCTCCCGCCATAAAAACTCATGTTCAAGA	65.10	0.40	SEQ ID NO: 29
	CCTCCCGCCATAAAAACTCATGTTCAAGA	64.96	0.10	SEQ ID NO: 30
30	CTCCCGCCATAAAAACTCATGTTCAAGA	63.37	-0.10	SEQ ID NO: 31
	TCCCGCCATAAAAACTCATGTTCAAGA	62.86	-0.10	SEQ ID NO: 32
	CCCGCCATAAAAACTCATGTTCAAGA	60.47	-0.10	SEQ ID NO: 33
	CGCCATAAAAACTCATGTTCAAGA	57.98	-0.10	SEQ ID NO: 34
	CGCCATAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO: 35
35				

In one aspect of the present method, at least two parameters are determined wherein the parameters are poorly correlated with respect to one another. The reason for requiring that the different parameters chosen are poorly correlated with one another is that an additional parameter that is strongly correlated to the original parameter brings no additional information to the prediction process. The correlation to the original parameter is a strong indication that both parameters represent the same physical property of the system. Another way of stating this is that correlated parameters are linearly dependent on one another, while poorly correlated parameters are linearly independent of one another. In practice, the absolute value of the correlation coefficient between any two parameters should be less than 0.5, more preferably, less than 0.25, and, most preferably, as close to zero as possible.